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Previews

RB from a Bud's Eye View

RB and related proteins block transcriptional activation of genes critical to initiation of the cell cycle and suppress unwanted cell division. The circuitry controlling this response is generally conserved from humans to yeast, but no negative regulator like RB has been found in yeast. In this issue of *Cell*, two studies (Costanzo et al., 2004; de Bruin et al., 2004) reveal that Whi5 appears to play the role of RB in preventing precocious cell cycle entry in budding yeast.

The retinoblastoma tumor suppressor protein, RB, and the related proteins, p107 and p130, are linchpins of the metazoan cell cycle (Frolov and Dyson, 2004; Trimarchi and Lees, 2002). Early studies showed that DNA tumor viruses inactivate RB in order to induce S phase entry in nondividing host cells. Now it appears that either RB itself, or one of its upstream regulators, is compromised in virtually all human tumors. RB-related proteins prevent cell division in quiescent cells and during G1 of the normal cell cycle by binding to and inhibiting the E2F family of transcription factors. In response to appropriate mitogens, cells induce G1-specific cyclin-dependent kinases (Cdks) which hyperphosphorylate these repressors and cause their dissociation from the transcription complex. This derepression enables E2F proteins and their heterodimeric partners to activate transcription of the next wave of cyclins and other genes responsible for progression to S phase.

Initiation of the budding yeast cell cycle follows a remarkably similar path (Breeden, 2003). There is an early induction of G1 Cdk activity that activates a pair of heterodimeric transcription factors (SBF and MBF). These transcription factors induce the expression of the next wave of cyclins along with hundreds of genes involved in DNA synthesis and other early cell cycle events. However, while activators have been identified, there are no known repressors to play the part of RB (Cosma, 2002). This is despite the fact that colonial yeast is also under great pressure to restrain cell division in cells lacking sufficient nutrients. What unfolds in this issue of *Cell* (Costanzo et al., 2004; de Bruin et al., 2004) is the culmination of a committed and systematic search for the missing transcriptional repressor of the budding yeast cell cycle.

Budding yeast undergoes an asymmetric cell division in which the mother cell can be distinguished from the daughter by its larger size. However, this size difference diminishes in the next cycle due to a longer G1 phase in the daughter cell. Moreover, when cells are subjected to adverse nutritional conditions, G1 phase is prolonged. These and other observations, made 30 years ago, indicate that size homeostasis and cell division control take place in G1. However, the control over the timing of the G1 to S transition has remained obscure, due largely to the fact that neither timing nor cell size changes are amenable to genetic selections or biochemical probings.

Mutations in the budding yeast G1 cyclin, Cln3, speed up the cell cycle and lead to a noticeably smaller cell size. If RB exists in yeast, its inactivation should also cause a reduced cell size. However, no good candidates were found until a library of yeast strains carrying deletions in every gene was generated. It was not long before two groups had screened this library and identified *whi5* for its unusually small cell size (Jorgensen et al., 2002; Zhang et al., 2002). *WHI5* is a previously uncharacterized gene with no revealing homologies, but *whi5* mutants are resistant to G1 arrest and their small cell size depends upon the primary E2F analog, SBF (Jorgensen et al., 2002). Now the Andrews, Tyers, and Wittenberg groups uncover further parallels between this interesting protein and RB-related proteins as antagonists of the transcriptional cascade that leads to cell cycle entry (Costanzo et al., 2004; de Bruin et al., 2004).

The initial genetic data indicated that Whi5 is a negative regulator acting upstream of SBF. These new studies demonstrate that loss of *WHI5* suppresses the G1 delay and large size of cells lacking the activator, Cln3. Conversely, excess *WHI5* is sufficient to prolong G1 and confer a large size to wild-type cells and is lethal in Δ*cln3* cells. These data are consistent with the view that Cln3/Cdk relieves inhibition of SBF by Whi5, just as the metazoan cyclin D/Cdk relieves inhibition of E2F by RB proteins.

These investigators went on to demonstrate an interaction between Whi5 and SBF, which requires both subunits of SBF (Swi4 and Swi6). Moreover, they showed that Cln3/Cdk phosphorylates Whi5, and promotes its dissociation from SBF. Using chromatin immunoprecipitation, they detected a transient association of Whi5 with SBF DNA complexes. Costanzo et al. (2004) used purified components to show that these interactions are direct, and de Bruin et al. (2004) showed that release of Whi5 is temporally correlated with activation of SBF-regulated transcription.

Whi5 has twelve potential Cdk phosphorylation sites and it is a direct target of the yeast cyclin-dependent kinase in vitro (Ubersax et al., 2003). de Bruin et al. (2004) found evidence of phosphorylation at five of those sites in vivo with mass spectrometry. Both groups mutated the Cdk sites, and assayed the consequences by different means. Costanzo et al. (2004) found that Whi5 is excluded from the nucleus from late G1 until mitosis. Mutating six of the potential Cdk sites causes nuclear retention of Whi5 throughout the cell cycle. However, this mutant has no effect on G1 progression. Even overproduction of a *whi5* mutant lacking all twelve Cdk sites causes a minimal phenotype. Consistent with this, their in vitro studies showed that Cdk activity releases Whi5 from SBF even when no Cdk sites are present in Whi5. These results suggest that Whi5 phosphorylation is not critical for derepression and that there may be other important Cdk targets in SBF. One plausible target is Swi6, because when the Cdk sites were eliminated from both Whi5 and Swi6, strain viability was lost.

In contrast, the Wittenberg lab found that Whi5 phosphorylation can play a role in regulating G1 progression.

They followed elutriated daughter cells ectopically expressing a *whi5* mutant with seven Cdk sites removed and observed a considerable extension of G1. This work was done in a different strain background, and cannot be directly compared to the experiments of Costanzo et al (2004). As a result, the detailed mechanism by which Cdk activity neutralizes the inhibitory function of Whi5 remains uncertain, but these complexities do not diminish the importance of Whi5 as a negative regulator. Rather, they remind us that cells do not rely on a single regulatory mechanism to keep their cell cycle in order. Moreover, while the general outline of cell cycle control is remarkably conserved, cells can differ with respect to which regulatory steps play the most prominent roles.

In summary, these studies extend the parallels between yeast and metazoans at the initiation of the cell cycle. Whi5 and the RB-like proteins show remarkable similarity both in their activity as repressors and in the regulation of that activity by Cdk-dependent phosphorylation. However, like the activators of transcription, there is no primary sequence homology between Whi5 and RB. Rather, these parallels serve as an exquisite example of convergent evolution and irrefutable testimony to the logic of this circuitry. They also illustrate the power of comparative studies in diverse model organisms for expediting a full understanding of any fundamentally conserved process.

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Catching Some Zs: A New Protein for Spatial Regulation of Bacterial Cytokinesis

Prior to its duplication, the bacterial nucleoid exerts local negative control over assembly of the cytokinetic Z ring to prevent potential cutting of the chromosome. In this issue of *Cell*, Wu and Errington show that a specific nucleoid-associated protein mediates this nucleoid occlusion effect, providing the first mechanistic insight into this key spatial regulatory system.

The choice of where to divide is a fundamental problem for most cells and determines the fate of progeny cells. In the case of rod-shaped prokaryotes such as *Escherichia coli* or *Bacillus subtilis*, the positioning of the cytokinetic ring at the cell midpoint is negatively regulated by the centrally positioned nucleoid. This effect, called nucleoid occlusion (NO), prevents unwanted cutting of an unsegregated chromosome by the cell division septum (Woldringh et al., 1990). The primary target of NO appears to be the tubulin-like FtsZ protein, which forms the cytokinetic Z ring. In mutant cells defective for chromosome segregation or replication initiation, the Z ring localizes to the edge of the nucleoid, far from the cell center (Harry, 2001; Margolin, 2001). This remarkable mislocalization indicates that the Z ring can potentially assemble anywhere in the cell but responds to negative effectors by assembling where the negative effect is lowest.

Another negative spatial regulator of cytokinesis is also found in many bacteria. Consisting of MinC, MinD, and MinE or DivIVA proteins, the Min system prevents unwanted Z ring assembly in nucleoid-free areas at the cell poles (Migocki et al., 2002). In Min^- cells, Z rings form at all DNA-free areas of the cell, including correct positions between daughter nucleoids and incorrect positions at the cell poles, but do not form over nucleoids because of NO (Yu and Margolin, 1999). Min^- cultures are viable because sufficient septation occurs medially to produce many daughter cells with chromosomes. The Min system exerts its effect via MinC, which locally inhibits FtsZ polymer assembly. This inhibition is spatially controlled by the action of the MinD protein, the majority of which is mainly restricted to the polar portion of the cell membrane by MinE in *E. coli* (Raskin and de Boer, 1999) or DivIVA in *B. subtilis* (Marston et al., 1998).

The currently accepted model for Z ring placement is that prior to chromosome segregation, the independent action of NO and Min-mediated inhibition results in the masking of all points on the cell membrane for Z ring assembly. Only when NO is relieved at the cell center during chromosome segregation is the medial Z ring allowed to assemble. Unfortunately, up to now, the mechanism behind NO has remained mysterious. Local DNA concentration appears to be an important mediator because conditions that reduce chromosome condensation result in suppression of NO, with Z rings often assembling on top of nucleoids (Margolin, 2001). The stage of chromosome replication is also an important factor (Harry, 2001). However, a molecular handhold has been needed for some time to gain a better understanding of how NO works.

A breakthrough has come with the discovery by Wu and Errington (2004) of a protein that mediates NO in *B. subtilis*. They found, serendipitously, that a mutant in *yyaA*, previously shown to have no discernible phenotype, was lethal when combined with a *min* mutant. To determine the cause of this lethality, they found that when both *yyaA* (subsequently called *noc* for "nucleoid occlusion") and *min* were inactivated, cells stopped dividing. Instead of forming rings exclusively between